Identification of Potential Targets of Stress Cardiomyopathy by a Machine Learning Algorithm

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Abstract

Background: Stress cardiomyopathy (SCM) is a reversible, self-limiting condition that manifests as left ventricular insufficiency. The incidence of stress cardiomyopathy has increased because of increasing mental and social stress, but the exact pathophysiological mechanisms remain unclear.

Methods: To elucidate the critical molecules in the pathogenesis of SCM and the functional changes that they mediate, we downloaded data for a healthy control group and stress cardiomyopathy (SCM) group from the Gene Expression Omnibus database, performed differential analysis, and analyzed the results of GO and KEGG enrichment analysis to describe SCM-associated genes and functions. Lasso, random forest, SVM-RFM, and Friends analysis were used to screen hub genes; CIBERSORT and MCPcounter were used to explore the relationship between SCM and immunity; and an animal model of SCM was constructed to conduct bidirectional verification of the obtained results.

Results: In total, 21 samples (6 healthy, 15 SCM) were used in this study. Overall, 39 DEGs (absolute fold change ≥ 1; P < 0.05), including 23 upregulated and 16 downregulated genes in SCM, were extracted. Three common hub genes (PLAT, SEMA6B, and CRP) were finally screened. We further confirmed that functional changes in SCM were concentrated in immunity and coagulation functions.

Conclusion: Three key genes (PLAT, SEMA6B, and CRP) in SCM were identified by machine learning, and the major functional changes leading to SCM, and relationships of SCM with immunity, were identified.

Keywords: stress cardiomyopathy; machine learning; hub gene; co-expression network; immune infiltration; stress cardiomyopathy rat model

Introduction

Stress cardiomyopathy is a transient, reversible heart failure syndrome. Because of its self-limiting clinical manifestations, it was initially considered benign. However, it is now associated with cardiac arrhythmias [1]. Intrinsic shock is closely associated
with stress cardiomyopathy and represents one of its severe complications. The incidence of stress cardiomyopathy has increased because of increasing mental and social stress. Understanding of the disease has deepened, but the exact pathophysiological mechanism remains unclear, thus posing tremendous challenges in clinical diagnosis and treatment [2].

The clinical symptoms of SCM closely resemble those of acute myocardial infarction. While coronary angiography can distinguish between these conditions, performing coronary angiography on SCM patients poses procedural risks without providing any therapeutic benefits [3]. Basic research on SCM has not solved the above problems, and the most commonly accepted theory of SCM remains catecholamine-induced myocardial injury [4]. The relevant clinical markers are TNI, B-type natriuretic peptide (BNP), and NT- proBNP. The lack of basic research and the singular focus of disease research may be the reasons for the difficulties in clinical diagnosis and treatment of SCM.

Previous findings have suggested that commonly used clinical cardiac biomarkers such as cardiac troponin T or I, creatine kinase isoenzyme, and BNP may have roles in the early clinical diagnosis of SCM. However, in biomarker validation in SCM and acute coronary syndrome (ACS) with clinical diagnostics, the indicators were found to lack accuracy [5]. Therefore, a biomarker for identifying stress cardiomyopathy is of high practical interest and might enable coronary angiography to be avoided in patients with low-risk SCM, thus avoiding exposure of patients to procedural risk [6].

At present, with the development of machine learning, new applications of machine learning algorithms in the medical field are emerging [7]. By combining machine learning algorithms with gene expression data, researchers can more efficiently and accurately discover molecular markers that play major roles in diseases, thus substantially advancing the mechanistic study of diseases [8].

However, few studies have used machine learning algorithms in stress cardiomyopathy to improve the accuracy of diagnostic markers obtained from screening; our study attempts to fill this gap [9]. Essential genes and pathways were identified through machine learning algorithms, to improve understanding of the pathophysiological mechanisms of disease development. We constructed a rat model of stress cardiomyopathy to verify our results. Clarifying the changes in crucial molecular markers and their functions in this disease may provide new ideas, and support the development of treatment modalities for clinical diagnosis and clinical treatment of SCM.

Materials and Methods

Data Sources
We selected 15 patients with stress cardiomyopathy data and 15 healthy control data from the GSE95368 dataset [9].

Data Preprocessing and Identification of DEGs
The workflow of the analysis is shown in Figure 1. The expression data, as well as the annotation files, were downloaded; the probes of the expression matrix were converted to gene symbols according to annotation files; log2 transformation of the expression matrix was performed; and the limma [10] function package in R software was used to compare gene expression between SCM and control samples, identify DEGs, and perform statistical analysis. The first level screening criteria for DEGs were P-value < 0.05 and | log2 fold change | ≥ 1. The second level screening criteria for DEGs were P-value < 0.05 and | log2 fold change | ≥ 2.

Enrichment Analysis to Explore Biological Functions
Enrichment analysis was conducted with the ClusterProfiler package [11], including GO enrichment, KEGG enrichment, and KEGG gene set enrichment analysis. Gene set variance analysis (GSVA) was conducted with the GSVA package, and the enrichment scores of these gene signatures were calculated [12].

Biomarker Identification with Machine Learning Algorithms
The selection of genes with potential diagnostic value through a machine learning algorithm was conducted in four parts. In the first part, we used the RandomForest package to perform gene screening
with the random forest algorithm to obtain the first part of biomarkers with potential diagnostic value [13]. In the second part, we used the glmnet package to perform regularization-based gene screening and then obtain the second set of biomarkers with diagnostic value [14]. In the third part, we used the SVM_RFE process for gene screening to obtain the third set of biomarkers [15]. Finally, we used Friends analysis to obtain the last set of biomarkers with diagnostic value [16]. We then removed the duplicates from the genes in the four sets, determined the union, and combined the results of the previous enrichment analysis to obtain candidate genes for subsequent bidirectional confirmation.

Immune Infiltration
To explore the relationship between SCM and immunity, we used CIBERSORT [17] and MCPcounter [18] for analysis of the immune infiltration of the expression matrix, and the rank sum test to analyze the proportions of identical immune cells in different groups [19].

Construction of a Rat Model of Stress Cardiomyopathy
Male Sprague-Dawley rats weighing 250–300 g were selected for immobilization stress for 6 hours over 7 days to establish an SCM model. The anesthetized rats were randomly divided into two groups: normal control and SCM. Ventricle tissues were extracted for RNA sequencing. All animal work was performed in accordance with the NIH guidelines for the use of animals in experiments.

Homologous Mapping
We used the BioMart package [20] to perform gene conversion between humans and rats, classify the genes according to the size and sign of logFC after conversion, and display the classification results in a set graph.

Construction of PPI Networks and Single-Gene Enrichment Analysis
After use of the machine learning algorithm and homologous localization of genes, we selected the up-regulated genes with the same logFC direction as input data. We then used the STRING database for PPI network construction [21].

Statistical Methods and Software
We used R version 4.2.1 and the rank sum test to compare the ratio of immune cells between groups.
The rest of the analysis was performed according to standard process for relevant R packages.

**Results**

**Identification of DEGs in SCM**

A total of 1228 differentially expressed genes were identified and visualized in volcano plots. Through the first screening on the basis of P value and logFC (P value less than 0.05 and logFC absolute value greater than 1), we obtained 23 differentially up-regulated genes and differentially down-regulated genes. Subsequently, we performed a second screening on genes on the basis of P value and logFC (P value less than 0.05 and logFC absolute value greater than 2), and obtained five differentially up-regulated genes and three differentially down-regulated genes (Figure 2).

**Enrichment Analysis**

GO enrichment analysis was performed on 43 important DEGs by using the ClusterProfiler package; the threshold Q value was set to <0.05. Figure 3A shows the analysis results of GO enrichment in terms of molecular function, biological process, and cellular component. The GO enrichment analysis results between stress cardiomyopathy and normal tissues identified functions related to humoral immunity (humoral response), leukocyte activation and proliferation, and platelet alpha granule. The results reflect activation of the immune system and alterations in the coagulation system, thus potentially explaining the clinical symptoms of SCM. For example, thrombosis in the left ventricle may occur.

Simultaneously, we performed KEGG pathway enrichment analysis on these DEGs (Figure 3B). The results indicated that the B cell receptor signaling pathway, lipid and atherosclerosis, and platelet activation play a significant role in SCM. The above findings suggest that clinical patients with SCM may show changes in the immune system and coagulation system, and support that SCM cannot be excluded in patients with previous coronary atherosclerosis. The results of the KEGG enrichment analysis of patients with SCM suggested the potential existence of atherosclerosis during the disease course.
Gene Set Enrichment Analysis and Gene Set Variation Analysis

Using the ClusterProfiler package, we performed GSEA on all DEGs. DEGs in stress cardiomyopathy were enriched in the functions of platelet activation, neural tissue interaction, lipid and atherosclerosis, and immune-related pathways. Because the intrinsic functional changes determine the clinical presentation, patients’ mild fever, left ventricular thrombosis, and chest pain might be explained by the enrichment results. The genes enriched in each pathway are visualized in Figure 3C.

We additionally sought to investigate whether the altered pathway functions were activated or inhibited. Therefore, we performed GSVA to explore differential pathways in stress cardiomyopathy. The up-regulated relevant pathways included ESTROGEN_RESPONSE_EARLY, HEME_METABOLISM, IL2_STAT5_SIGNALING, and COAGULATION (Figure 3D). These results suggested activation of the immune response in SCM, thus potentially indicating that the pathogenesis of stress cardiomyopathy may be immune-related; activation of coagulation mechanisms, thus potentially suggesting that patients with stress cardiomyopathy have thrombosis, which may be a potential factor contributing to poor patient prognosis; and the importance of early anticoagulation. Furthermore, our findings suggest that changes in estrogen levels may be associated with a higher incidence of stress cardiomyopathy in women than in men. A more in-depth discussion of the results is provided in the Discussion section.

Immune Infiltration Analysis

First, we used two immune infiltration methods to estimate the infiltration of immune cells between the SCM group and normal control group, and plotted the results in an integrated heat map (Figure 4A). The results suggested differences in stress cardiomyopathy at the immune level, which are elaborated upon in the Discussion section. To demonstrate the differences in the proportions of the same cell types among subgroups, we constructed violin plots to show the full results. Our findings suggested differences in the proportions of monocytes and T cells in
stress cardiomyopathy, as further elaborated upon in the Discussion (Figure 4B).

**Screening of Potential Biomarkers**

We used four algorithms to screen the best biomarkers for diagnosing SCM. One gene was identified with the LASSO regression algorithm, 20 genes were identified with the SVM-RFE algorithm (Figure 5A), 20 genes were identified with the random forest algorithm (Figure 5B), and 20 genes were identified with Friends analysis (Figure 5C). The candidate markers obtained with the machine learning algorithm were summarized and used in subsequent analyses.

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**Figure 4**  (A) Use of CIBERSORT and MCPcounter, two immune infiltration methods, to reveal the immune characteristics of SCM. (B) Rank sum test based on the proportion of immune cells.

**Figure 5**  (A) Screening genes with potential predictive value with the SVM-RFE algorithm. (B) Screening genes with the random forest algorithm. (C) Friends’ analysis identification of hub genes in the expression matrix.
Differential and Enrichment Analysis of Restraint Stress Rat Models

GO enrichment analysis was performed after the differential analysis in the SCM rat model (Figure 6A). Restraint stress model rats exhibited functionally similar results to the previous human data. Subsequently, we performed KEGG enrichment analysis to further validate our previous conclusions (Figure 6B). We then visualized the relationships between functions and genes (Figure 6C), to guide the final selection of SCM biomarkers. The results of the enrichment analysis in the rat model of stress cardiomyopathy supported the previous results indicating primarily immune and coagulation disease mechanisms. The findings also confirmed the conclusions’ reliability and were highly reproducible after validation in animal studies. The relevant functions are discussed in detail in the Discussion.

Homology Mapping and Elucidation of Biological Functions

Next, we obtained biomarkers of interest, clarified their functions in SCM, and explored their relationships with immunity. Simultaneously, we explored the rat model of stress cardiomyopathy. Subsequently, we narrowed the scope and verified whether the biomarkers were accurate. The first portion of the results was from humans, and the second portion of the results was from rats. We next performed homology mapping, and chose biomarkers with the same logFC direction for subsequent use as biomarkers for SCM. The above results were plotted in a set graph (Figure 7A).

After homology mapping, converting rat genes to human genes, and selecting the same logFC direction in both datasets, we finally obtained markers with diagnostic value, including those that were down-regulated in the same direction (PRLR, DPP7, SRC, RAD51, APOB, MAPK11, NAMPT, PTPN6, NCK1, LTA4H, POMC, LYN, FYN, UCHL1, C3, VAV1, EHMT2, TP53, GPC6, ICOSLG, and PRKACA) and up-regulated: (PLAT, SEMA6B, and CRP). We were more interested in genes that were up-regulated in SCM. We next performed single-gene enrichment analysis of these two groups of genes to clarify their functional changes, and constructed PPI networks to observe the relationships among genes. First, we performed a correlation analysis on the genes SEMA6B and PLAT based on the entire expression matrix,

Figure 6  (A) GO enrichment analysis. (B) KEGG enrichment analysis. (C) Circle diagram showing the relationships between genes and functions.
selected a gene set with a correlation coefficient greater than 0.6, and then performed GO enrichment analysis (Figure 7B) and KEGG enrichment analysis (Figure 7C) on the gene set. The PPI network of PLAT (Figure 7D), the PPI network of SEMA6B (Figure 7E), and the PPI network of CRP (Figure 7F) were constructed to provide mechanistic insights for the clinical diagnosis and treatment of SCM.

Discussion

Although stress cardiomyopathy is increasingly known and accepted, the mechanism of the disease is not fully understood.

In this study, we identified gene expression differences between diseased and healthy states, including related functions and pathways, through differential gene expression analysis in blood samples from individuals with stress-induced cardiomyopathy and healthy individuals. We used four machine learning algorithms – the LASSO regression algorithm, SVM-RFE algorithm, random forest algorithm, and Friends analysis – to identify biomarkers with potential diagnostic value. Through the use of animal models for preliminary validation, we identified truly diagnostic biomarkers either down-regulated in the same direction (PRLR, DPP7, SRC, RAD51, APOB, MAPK11, NAMPT, PTPN6, NCK1, LTA4H, POMC, LYN, FYN, UCHL1, C3, VAV1, EHMT2, TP53, GPC6, ICOSLG, and PRKACA) or up-regulated (PLAT, SEMA6B, and CRP). We performed single-gene enrichment analysis and PPI network construction on the up-regulated genes or particular interest. The obtained results may aid in mechanistic research and clinical diagnosis of stress cardiomyopathy.

Through the differential analysis between the SCM and control groups, we identified 1228 DEGs. Through the first screening according to P value and logFC (P value less than 0.05 and absolute value of logFC greater than 1), we obtained 23 up-regulated DEGs and 16 down-regulated DEGs. Subsequently, we performed a second screen on the basis of P value and logFC (P value less than 0.05 and absolute value of logFC greater than 2), and obtained five up-regulated DEGs and three down-regulated DEGs (Figure 2). Among these genes, GAPDH [22] and NPPB [23] have known relationships with nerves and the heart, according to previous studies. A very important hypothesis in stress cardiomyopathy is the interaction of the heart and the brain; therefore, this result appears reasonable [24].
The results of GO enrichment analysis also indicated that the functions of DEGs were concentrated primarily in platelet activation, neural tissue interaction, lipid and atherosclerosis, and immune-related functions (Figure 3A). The function platelet activation corroborated the clinical manifestation of intra-left ventricular thrombosis in stress cardiomyopathy and provides a basis for the use of antiplatelet agents [3]. The function lipid and atherosclerosis is reflected in stress cardiomyopathy; this finding appears reasonable, because previous studies have shown that, even if a clinical patient has coronary atherosclerosis or has had acute myocardial infarction, the possibility of stress cardiomyopathy cannot be directly ruled out. Therefore, the use of lipid-lowering drugs is warranted [24]. Notably, immune-related functions are represented in stress cardiomyopathy. The roles of immune responses in stress cardiomyopathy are far more complex than previously understood, and recent studies support our conclusions [25]. Subsequent immune infiltration analysis will elaborate on immune roles in stress cardiomyopathy.

The results of KEGG enrichment analysis partly corroborated the results of GO enrichment analysis but partly also corroborated another possible mechanism of stress cardiomyopathy (Figure 3B). The enrichment in the PI3K-Akt signaling pathway indicated the role of catecholamine release in stress cardiomyopathy; the application of inhibitors of this pathway may decrease apoptosis due to oxidative stress in stress cardiomyopathy [26]. Meanwhile, the role of the JAK-STAT signaling pathway in regulating oxidative stress and immune response has been widely studied [27]. Recent studies have found that the JAK-STAT signaling pathway affects the prognosis of septic cardiomyopathy [28]. However, research on the role of stress cardiomyopathy is lacking and therefore will be one of the directions of our follow-up research.

Because the enrichment analysis based on the ORA algorithm can only locate the function but cannot explain whether the obtained function is up- or down-regulated, we performed GSVA. We obtained genes related to the pathway of interest through GSEA. The results of GSVA may explain some of the clinical manifestations of stress cardiomyopathy (Figure 3D), such as the upregulation of the ESTROGEN RESPONSE EARLY function, thus suggesting that sex influences stress cardiomyopathy [24]. Interestingly, the upregulation of HEME METABOLISM and GLYCOLYSIS suggested that myocardial hypoxia and increased peripheral myocardium metabolism due to stress cardiomyopathy do indeed occur [29]. The upregulation of immune-related functions (IL2 STAT5 SIGNALING) and coagulation-related functions (COAGULATION) suggested that the belief that stress cardiomyopathy is self-limiting may be inaccurate. The duration of myocardial damage may be much longer than anticipated [25].

To further explore the relationship between stress cardiomyopathy and immunity, we used two immune-infiltrating analysis methods, CIBERSORT and MCPcounter. Interestingly, the results of the immune infiltration analysis suggested that macrophages are associated with the immune mechanism in stress cardiomyopathy. Although limited by the sample size, an apparent change in M1-phase macrophages, but not in M2-phase macrophages, was broadly observed (Figure 4A). This finding was notable because, in single-cell sequencing studies of immune cells in myocardial infarction, 1 day after myocardial infarction, macrophages show a substantial decrease followed by an increase, and the transition from M1 to M2 is relatively clear [30]; however, in the case of stress cardiomyopathy, the transition between M1 and M2 is less smooth, thus potentially suggesting that the disease cycle is not short. Stress cardiomyopathy has been demonstrated to be distinct from myocardial infarction in terms of macrophage changes, and our findings further enhance their precision [31].

The rank sum test was then performed on the expression in the same cell type in different samples (Figure 4B). The proportion of T cells differed significantly within various subgroups. In contrast, the proportion of B cells did not differ significantly within subgroups, thereby indicating prolonged inflammation duration during the progressive phase of the disease [32]. Interestingly, a review of past case studies revealed that patients treated with CAR-T in oncology therapy show manifestations similar to those of stress cardiomyopathy [33], thus suggesting that surface T cells may be involved in the development of stress cardiomyopathy and that early treatment with immunosuppressive agents does improve the prognosis of stress cardiomyopathy [34]. These findings may suggest a unique role of immunity in stress cardiomyopathy. According to the literature, myocardial edema often begins to dissipate after long time periods [31].
In addition, substantial changes in the proportion of monocytes occur in stress cardiomyopathy, and past studies have suggested that, in myocardial infarction, macrophages are uniformly distributed throughout the myocardium in early stages, whereas monocytes infiltrate the infarcted area [30]. Given that stress cardiomyopathy and acute myocardial infarction have similar clinical manifestations, we propose a hypothesis in which stress cardiomyopathy may have similar manifestations to acute myocardial infarction at the immune level, including early infiltration of monocytes in the apical region. No studies have compared stress cardiomyopathy with acute myocardial infarction. Our hypothesis will be the focus of our subsequent studies.

We sought to explore biomarkers with potential diagnostic value in SCM. Given that machine learning is currently increasingly applied in the medical field [7], and machine learning algorithms for feature selection have shown good performance in the medical field [35], we filtered our expression matrix by using four algorithms for screening genes (Figure 5). A total of 53 biomarkers with potential diagnostic value were identified.

The purpose of the enrichment analysis in the rat model of stress cardiomyopathy was first to verify the model’s accuracy and second to obtain more realistic results while controlling for confounding factors (Figure 6). The results of the GO enrichment analysis were similar to those of the GO enrichment analysis described above, thus indicating the accuracy of the rat model of stress cardiomyopathy. The results of KEGG enrichment analysis identified the PPAR signaling pathway, cytokine-cytokine receptor interaction, and other pathways, which are described separately below.

PPAR signaling pathway describes a class of pathways that contain many subtypes, including PPARα, PPARδ, and PPARγ. Its function in cardiovascular disease alters lipid levels and muscle fiber status and is susceptible to external stimuli. Hence, this pathway contributes to stress cardiomyopathy related functional changes [36].

Cytokine-cytokine receptor interaction has long been studied in stress cardiomyopathy, and our results support such study at the level of data analysis [37].

The PI3K–Akt signaling pathway has been studied in coronary artery disease and found to modulate disease progression primarily through the regulation of oxidative stress [38]. Our study further provided the first demonstration that this signaling pathway is also present in stress cardiomyopathy and is involved in disease progression.

However, using only algorithms to screen biomarkers may lead to biased results. Therefore, we confirmed the candidate diagnostic markers in a rat model of stress cardiomyopathy. The gene symbols of the candidate diagnostic markers in rats were obtained by homology mapping, and the candidate diagnostic markers with the same logFC direction in humans and rats were selected as real diagnostic markers (Figure 7A).

In this way, we obtained truly diagnostic biomarkers, including those down-regulated in the same direction (PRLR, DPP7, SRC, RAD51, APOB, MAPK11, NAMPT, PTPN6, NCK1, LTA4H, POMC, LYN, FYN, UCHL1, C3, VAV1, EHMT2, TP53, GPC6, ICOSLG, and PRKACA) and up-regulated (PLAT, SEMA6B, and CRP). The single-gene enrichment analysis (Figure 7B) and the construction of PPI networks (Figure 7D) were performed on the genes that were up-regulated in the same direction. The enriched results also suggested that, in stress cardiomyopathy, the functional changes were concentrated primarily in two aspects: coagulation and immunity. The specific changes to the relevant functions were explained above.

Among screened molecules, the SEMA6B gene has been extensively studied in epilepsy [39]. In cases of epilepsy leading to death, patient pathological sections of the heart are highly similar to those of patients with stress cardiomyopathy [24], thus suggesting that this gene is likely to play multiple roles. On the basis of the available results, this gene can reasonably be concluded to act as a trigger similar to that of stress cardiomyopathy.

The PLAT gene is differentially expressed in stroke [40], and the cardiac pathological sections of patients who die from stroke, subarachnoid hemorrhage, and other cerebral circulation disorders are similar to those of patients with stress cardiomyopathy [24]. Recent studies have begun to investigate the association of PLAT with neurological death in the brain [41]. The discovery of this gene in stress cardiomyopathy may facilitate the exploration of the disease from a different perspective.
CRP genes are differentially expressed in tissue injury, infection, or other inflammatory stimuli [42], and CRP is now known to be widely present in various immune response diseases [43]. According to our results, the immune mechanism of stress cardiomyopathy is complex and distinctive, and the CRP gene can be used to study the immune mechanism of stress cardiomyopathy.

Simultaneously, because we focused on up-regulated genes, we did not explore many down-regulated genes, i.e., altered biological mechanisms elicited by loss of function, because we considered that studying altered biological mechanisms elicited by overexpression of genes might be more meaningful in conjunction with the causative mechanism of stress cardiomyopathies. Therefore, we focused on the altered function of up-regulated genes. Simultaneously, owing to the limitation of experimental conditions, we did not conduct relevant basic experiments for verification; however, we used a rat model of stress cardiomyopathy to compensate for this limitation.

Conclusion

Three key genes (PLAT, SEMA6B, and CRP) in SCM were identified through machine learning, and the major functional changes leading to SCM and the relationships of SCM with immunity were identified.

Ethics Statement

All methods were conducted in compliance with pertinent guidelines and regulations.

Data Availability Statement

All raw data are from the Gene Expression Omnibus database and can be downloaded via GEO number GSE95368. On reasonable request, relevant animal model data can be obtained by contacting the authors. Relevant codes used for data analysis are available from https://github.com/Jxuanrui/SCM_MachineLearrning.

Author Contributions

Xuexin Jin and Xuanrui Ji: conceptualization (equal); data curation (equal); writing – original draft. Junpei Zhang and Hongpeng Yin: methodology (equal); project administration (equal); formal analysis (equal); writing – original draft (equal). Pengqi Lin and Quanwei Pei: investigation (equal); project administration (equal). Junpei Zhang, Bin Li, and Dezan Su: investigation (equal); project administration (equal). Xiufen Qu and Dechun Yin: project administration (equal); Wei Han: conceptualization (lead); supervision (lead); validation (lead); Xuanrui Ji: investigation (equal); project administration (equal); writing – original draft (equal); writing – review and editing (equal).

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Conflict of Interest

The authors have no financial conflicts of interest to disclose concerning this study.

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